TABLE III. A Representative Example of a Neutralization Test Employing the Virus Dilution Technic.

Serum samples	Virus dilutions 1/50	1/100	1/200	1/400	1/800	1/1600	1/3200	ID_{50} titer log of virus dilution	Neutralization index
N*F89	—	4/4†	4/4	3/5	3/5	0/5	0/5	2.8	
I F89	2/4	2/5	0/4	0/4	0/4	0/5	—	1.8	10

* N = Normal; I = Immune.
† Numerator indicates No. of eggs showing lesions. Denominator indicates total No. of eggs.

tibody in human gamma globulin and some samples of human serum was also demonstrated. The significance of these findings can not be determined at the present time. A possible direct relationship between canine distemper and a respiratory disease of humans has been suggested by Adams(4).

Summary. Virus-neutralization tests were carried out in embryonated hen's eggs employing the egg-adapted distemper virus and various samples of serum. Ferrets whose sera contained no demonstrable antibody at 1:40 dilution all succumbed to 100 MLD of ferret distemper virus, whereas ferrets showing antibody titer to 1:640 dilution of serum all survived the same challenge dose. The data presented demonstrate that the results of serum-neutralization tests in chick embryos have a definite correlation with ferret protection tests. Human gamma globulin and some samples of human serum have neutralizing substances in titers as high as those known to exist in immune ferret serum. On the other hand, samples of serum from normal premature infants did not show any neutralizing substances in 1:40 dilution of the serum.

TABLE IV. Results of Serum-Virus Neutralization Tests in Eggs, Employing the Virus Dilution Technic.

Serum samples	Log ID_{50} titer of virus: Control serum	Immune serum	Neutralization index
F85	3.2	2.1	13
F86	3.0	1.9	13
F89	2.8	1.8	10
F88	3.0	1.8	16
Human gamma globulin		1.4	
Canine hyperimmune serum		.7	

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Effect of Dicumarol on Localized Shwartzman Reaction.* (21267)

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There have been numerous theories as to the pathogenesis of the Shwartzman reaction. One theory is that it is caused by excessive concentration of toxin in the prepared site after the intravenous or provocative injection due to a localizing effect of the inflammatory reaction. This would appear to be contradicted by the fact that local injection of large amounts of toxin into the skin does not pro-

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duce a dermal Shwartzman reaction. Another theory is based on the fact that skin tissues which have been prepared for the dermal Shwartzman are metabolically abnormal in that they produce large amounts of lactic acid aerobically(1). An additional finding is that rabbits given intravenous toxin become highly susceptible to the necrotizing effect of a proteolytic enzyme injected intradermally. On the basis of the preceding facts it was suggested that the local Shwartzman might be due to activation of a tissue protease in the skin. Stetson(3) suggested that the Shwartzman reaction was due to clumping of platelets and white cells occluding the vessels in the affected area and leading to hemorrhagic necrosis. The work of Good and Thomas(2) supports this theory. They found that heparin in doses large enough to prolong the clotting to 60 minutes or longer would prevent both the local and generalized Shwartzman reactions. Heparin, in addition to its anticoagulant action has effects on the proteins and lipids of the blood(4). However, Good and Thomas felt that since the action of heparin in preventing the Shwartzman reaction seemed to be closely correlated with its alteration of the clotting mechanism, it was most likely mediated through its effect on blood coagulation.

The present study was undertaken to elucidate further the action of anticoagulants on the Shwartzman reaction. It is generally agreed that heparin inhibits both the conversion of prothrombin to thrombin and that of fibrinogen to fibrin(5). Dicumarol inhibits the clotting mechanism by depressing the levels of prothrombin and factor VII in the blood. It was felt that by using dicumarol in place of heparin, it would be possible to see if the Shwartzman reaction could be inhibited by anticoagulants whose mechanism of action was different from that of heparin.

Materials and methods. Meningococcus toxin was prepared from meningococcus strain 44B,† cultured by heavy seeding on the surface of Mueller-Hinton starch agar in Kolle flasks and grown for 48 hours in candle jars. The growth in each bottle was washed off with approximately 4 cc of physiological saline. The resultant suspensions were then centrifuged for 30 minutes and the supernatant fluid was stored for 48 hours at 4°C. At the end of this time the material was cultured and was used only if the cultures were negative. The dermal Shwartzman reaction was produced by giving .25 cc of a 1:2 dilution of toxin intradermally. This was followed in 24 hours by an intravenous injection of 2 cc of a 1:30 dilution of toxin. The dermal site was then inspected for the presence or absence of hemorrhagic necrosis 5 hours after the provocative injection. The rabbits used were 1-2 kg hybrid albino rabbits, divided into 2 groups. One group served as controls and the other received dicumarol. The treated group received 50 mg of dicumarol daily till the prothrombin time was 40 seconds or over. They were then given the preparatory injection of toxin and 24 hours later, after the prothrombin time was again checked, they were given the provocative injection. They were then given the preparatory injection of toxin and 24 hours later, after the prothrombin time was again checked, they were given the provocative injection. It was found impractical to try to prolong the prothrombin time much over 40 seconds because of the hemorrhagic complications encountered.

† Obtained through the courtesy of Dr. Gregory Shwartzman, Mt. Sinai Hospital, New York City.

TABLE I. Control Group, 10 Rabbits.

Prothrombin time on 5/5, sec.	Result
10	Negative
12	Hem. necrosis
11	*Idem*
11	"
10	"
10	"
11	Negative
9	Hem. necrosis
9	*Idem*
9	"

On 5/5 all rabbits were given 0.25 cc of 1:2 dilution of meningococcus toxin intradermally. On 5/6, 24 hr later, 2 cc of a 1:30 dilution of toxin was given intravenously.

Results for 16 rabbits are in Tables I and II.

Discussion. It may be seen from these results that there was no significant difference between the treated and control groups, 5 of

TABLE II. Treated Group, 6 Rabbits.

Pro time value with date obtained		Date & time preparatory inj. given	Pro time value with date just before provocative inj.		Date provocative inj. given	Results
	Sec.			Sec.		
5/17 3 P.M.	40	5/17 6 P.M.	5/18 5 P.M.	51	5/18 6 P.M.	Hem. necrosis
Idem	55	*Idem*	*Idem*	105	*Idem*	*Idem*
"	50	"	"	90	"	"
"	45	"	"	50	"	"
"	44	"	"	40	"	Negative
5/18 10:30 A.M.	60	5/18 1 P.M.	5/19 1 P.M.	60	5/19 1:45 P.M.	Hem. necrosis

6 of the treated group and 8 of 10 of the controls showing hemorrhagic necrosis.

It would seem, therefore, that the effect of heparin in inhibiting the dermal Shwartzman reaction, if it is due to interference with the clotting mechanism, is not due to any property which it has in common with dicumarol. Since they both inhibit the formation of thrombin it would appear that if heparin is effective because of its anticoagulant activity, the site of action in inhibiting the dermal Shwartzman is by preventing the conversion of fibrinogen to fibrin. Another possibility is that the inhibition by heparin of the dermal Shwartzman is an additive one, effects being exerted on both the formation of thrombin and also on that of fibrin, the two effects together being necessary for the inhibition of the dermal Shwartzman reaction.

The results in the present work are similar to those in another study(6) in which dicumarol and heparin were compared with regard to their effect on clot formation in isolated venous segments. It was found that heparin inhibited clotting while dicumarol did not. In spite of dicumarol's ineffectiveness, bleeding complications were much more severe in the dicumarol than the heparin-treated animals.

Summary and conclusions. 1. The effect of dicumarol on the inhibition of the dermal Shwartzman reaction was studied. 2. It was found that unlike heparin, dicumarol did not inhibit the dermal Shwartzman reaction.

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Calcification XIV. Investigation of the Role of Chondroitin Sulfate in the Calcifying Mechanism.*† (21268)

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Mucopolysaccharides which contain sulfate are present in dentin and enamel(1), in bone (2), and in abnormal calcification of the arteries(3). Rubin and Howard(4) used meta-

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